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PROFILE OF FREE RADICAL SCAVENGING AND ANTIBACTERIAL ACTIVITIES OF EXTRACTS FROM *SCUTELLARIA BAICALENSIS* GEORGI RADIX

Li-Hong Zhang and Qing-Ping Hu*
College of Life Sciences, Shanxi Normal University, Linfen 041004, China

ABSTRACT: *Free radical scavenging and antibacterial activities of the extracts from the radix of Scutellaria baicalensis Georgi were examined in this paper. The extracts exhibited the better scavenging activities against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salts (ABTS). The results of antibacterial tests showed that the sensitivities to the extracts of different foodborne pathogens tested were different based on Oxford cup method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays, and the extracts exhibited better antibacterial activity against tested Gram-positive and Gram-negative bacterial. These results suggest that Scutellaria baicalensis Georgi may be a new potential source as a natural antioxidant and bacterial inhibitor.*

KEYWORDS: Free radical, Antibacterial activity, *Scutellaria baicalensis* Georgi

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism in living organisms. However, the excessive amounts of ROS and RNS are most responsible for the development of many diseases including carcinogenesis, atherosclerosis and heart diseases (Alexandrova & Bochev 2005; Valko *et al*., 2007). In addition, food poisoning and food spoilage caused by microorganisms are still the most important issues facing the food industry and consumers (Shan *et al*., 2007). For many years, a variety of different chemical and synthetic compounds has been made to prevent oxidation and control microbial growth (Li *et al*., 2012). However, consumers have grown concerned about the side effects of synthetic chemicals and want safer materials for preventing and controlling oxidation and pathogenic microorganisms in foods (Tiwari *et al*., 2009; Delgado-Adámez *et al*., 2012).

Plants can be an excellent source of natural antioxidants to preserve the quality and improve the shelf-life of food products (Tiwari *et al*., 2009; Voon *et al*., 2012). Use of natural plant derived antimicrobials can also be highly effective in reducing the dependence on antibiotics (Voon *et al*., 2012). In addition, plants or their extracts can also be used as natural colorants of foodstuffs; as in most of the cases, they are believed to be safe, and non-toxic to humans (Burt 2004; Rymbai *et al*., 2011).

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Scutellaria baicalensis Georgi is widely distributed through out the world, mainly in Asia, known as Huangqin in Chinese and Ogon in Japanese. The radix of *Scutellaria baicalensis* Georgi is a traditional herb medicine in China. It is a nontoxic nature product with multiple pharmacological effects and a complex chemical composition (Gao *et al*., 1999), ant it exhibits a wide range of biological activities, including antioxidative, antiinflammatory, antibacterial and antiviral activities (Gao *et al*., 1999; Ye *et al*., 2002; Duan *et al*., 2007; Kumagai *et al*., 2007; Park 2014 *et al*., 2014). However, few studies have investigated its free radical scavenging activity and antibacterial activities towards some foodborne pathogens. The objective of the present study was to investigate the DPPH and ABTS radical scavenging activity, and antibacterial effect of the radix of *Scutellaria baicalensis* Georgi extract on some foodborne pathogens.

MATERIALS AND METHODS

Materials and Regents: The radix of *Scutellaria baicalensis* Georgi was purchased from the local market. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salts (ABTS) were from Sigma. Nutrient agar (NA) and nutrient broth (NB) were from Beijing Aoboxing Bio-tech Co. Ltd. (Beijing, China). Other chemicals used were all of analytical grade.

Microbial Strains and Culture: The antibacterial activity was tested against six different microorganisms. Three Gram-positive strains were *Staphylococcus aureus* ATCC 25923, *Staphylococcus albus* ATCC 8799, and *Bacillus subtilis* ATCC 6051. Three Gram-negative bacteria were *Salmonella typhimurium* ATCC 19430, *Shigella dysenteriae* CMCC (B) 51252 and *Escherichia coli* ATCC 25922. The strains were provided by the College of Life Science, Shanxi Normal University, and cultured at 37 °C on NA or NB mediums.

Preparation of Extracts: The dried radix of *Scutellaria baicalensis* Georgi were finely ground with a micro plant grinding machine. Fifty gram of ground samples were blended with 500 mL methanol and shaken with a laboratory rotary shaker at 150 rpm for 5 h, and then the homogenates were centrifuged for 15 min at 4 ^{o}C and 5000 g. After centrifugation, the supernatants were pooled, and vacuum-evaporated to dryness at 40 ^{o}C, and reconstituted with solvent to a volume of 10 mL.

Determination of Total Phenolic Content: Total phenolic content was determined using the Folin-Ciocalteu colorimetric method as described by Rebey et al. (2012) with slight modifications. An aliquot (0.1 mL) of diluted extracts, 2.8 mL of deionized water and 0.1 mL of 1.0M Folin-Ciocalteu reagent were mixed and stirred. After 8 min, 2 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured at 765 nm wavelength after incubation for 2 h at room temperature.Total phenolic content was expressed as milligram gallic acid equivalent per gram dried weight

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(mg GAE/g DW).

Determination of Flavonoid Content: The level of flavonoid was measured as described by Rebey et al. (2012) with some modifications. Briefly, an aliquot (1.0 mL) of diluted extracts and 0.3 mL of 5% $NaNO_2$ solution were mixed for 6 min. Then 0.3 mL 10% $Al(NO_3)_3$ was added and incubated for 6 min. Next, 4 mL of 4% NaOH was added. The final volume was adjusted to 10 mL with distilled water and mixed thoroughly. After 15 min, absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Rutin was used for calibration of the standard curve and the content of flavonoids was expressed as milligram rutin equivalent per gram dried weight (mg RE/g DW).

DPPH Radicals Scavenging Activity Assay: DPPH radical scavenging activity was determined according to the method of Xu et al. (2012). The 2.5 mL of 0.6 mM DPPH methanol solution was added to 0.5 mL of sample solution of different concentrations to make the test solutions; the negative control (blank) consisted of 2.5 mL of DPPH solution plus 0.5 mL of methanol. These solutions were allowed to react at room temperature for 30 min in the dark, and the absorbance was measured at 517 nm. The scavenging rate of DPPH radical was calculated according to the formula given below: DPPH radical scavenging ability (%) = $[A_0-(A_1-A_2)]/A_0\times100$, where A_0 is the absorbance of the control in which methanol substitutes extract and A_1 is the result of the mixture of the extract and DPPH radicals while A_2 is the absorbance of the mixture of the extract and 2.5 mL methanol to eliminate the color effect of the extract.

ABTS Free Radicals Scavenging Activity Assay: This assay based on the inhibitionby antioxidants of the absorbance of the free radical cation from ABTS. ABTS was incubated with potassium persulfate in order to produce the free radical cation (ABTS). In brief, ABTS was dissolved in deionized water to make a 7 mmol/L concentration solution. ABTS was produced by mixing ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and the mixture was allowed to stand in the dark at room temperature for 12-16 h before use. In our study, the ABTS solution was diluted with methanol, to an absorbance of 0.70 (±0.02) at 734 nm. After addition of 3.8 mL of diluted ABTS to 100 µL of extracts, the absorbance reading was taken exactly 6 min after initial mixing.

Oxford cup method. Oxford cup assay was carried out according to the method described by Diao et al. (2013) with minor modifications. The extract was dissolved in methanol to prepare a 50% concentration which was then sterilized by filtration through 0.22 µm Millipore filters. The bacterial strains were incubated at 37 °C for 10 h on nutrient broth (NB) medium (The bacteria were incubated in the same condition for the following tests). The 200 µL of suspension containing 1×10^{6} colony forming units (CFU)/mL of bacteria were spread on nutrient agar (NA) medium. Oxford cups (6 mm in diameter) were placed on the inoculated agar, and then 200 µL of extracts was added with a micropipette. The diameter of inhibition zone (DIZ) was measured after 24 h of incubation at 37 °C.

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Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) assay. MIC and MBC were determined according to the method described by Diao et al. (2013) with minor modifications. Briefly, stock solution of the extracts was prepared in methanol. Two fold serial dilutions of extracts were filtered through 0.22 μm Millipore filters and prepared in sterile NB medium. To each tube 50 μL of the exponentially growing bacterial cells was added into medium, and the cell concentration in each medium was approximately 1×10^{6} CFU/mL. A control test was also performed containing inoculated broth supplemented with only methanol. The tubes were then incubated at 37 °C for 24 h and examined for evidence of the growth. The MIC was determined as the lowest concentration of the extracts that demonstrated no visible growth. The MBC was determined as follows. After the determination of the MIC, 100-fold dilutions with antimicrobial-free NB from each tube showing no turbidity were incubated at 37 °C for 48 h. The MBC was the lowest concentration of the sample that showed no visible growth in the antimicrobial- free cultivation. All experiments were performed in triplicate.

Kill-time analysis. The assay was performed according to the method described by Muroi and Kubo (1993) with some modifications. The effects of extracts with different concentrations (0.5×MIC, and 1×MIC) on the growth of tested bacteria were studied. Fifty microliters of the samples filtered through 0.22 μm Millipore filters was added to 4.9 mL of the sterile NB medium, and then mixed with 50 μL of a 10 h culture of tested bacteria (1×10^{6} CFU/mL). A control test was also performed containing inoculated broth supplemented with only methanol. The cultures were incubated at 37 °C and shaken with a rotary shaker at 120 rpm. In culture, seven samplings were carried out at 1, 2, 3, 5, 7, 9, 12 h, and the absorbance of sample was measured at 600 nm (OD_{600}).

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid Content: In this study, the levels of total phenolics from the radix of *Scutellaria baicalensis* Georgi was 8.13 mg GAE/g DW, while total flavonoid content was 2.21 mg RE/g DW (data not shown).

DPPH Radical Scavenging Activity: The scavenging activity assayed herein on DPPH radicals is shown in Figure 1. The scavenging activity of extracts increased significantly from 15.3% to 80% when its concentration increased from 2 mg/mL to 8 mg/mL. Thereafter, the scavenging activity had no obvious increase with the increase of concentration, which showed a concentration-dependent scavenging of the DPPH radicals at some concentrations.

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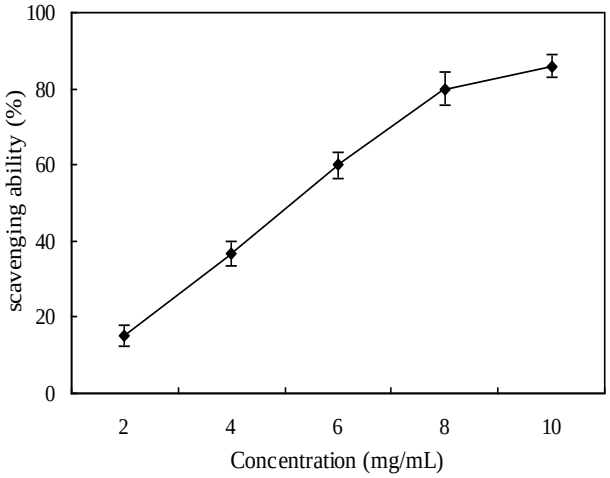

Figure 1. DPPH radicals scavenging activity of extracts

ABTS Radicals Scavenging Activity: The scavenging activity of extracts on ABTS radicals is shown in Figure 2. The profile of scavenging activity on ABTS was similar to the result of the scavenging activity on DPPH radicals. Somewhat differently, the scavenging activity on ABTS radicals increased significantly from 27.5% to 87.6% when its concentration increased from 20 mg/mL to 60 mg/mL. Thereafter, the scavenging activity had no obvious increase with the increase of concentration. Similarly, ABTS radicals scavenging activity of extracts increased dose-dependently at concentrations, which may be attributable to its hydrogen-donating ability. These differences in data between DPPH and ABTS assays were likely due to different experimental conditions.

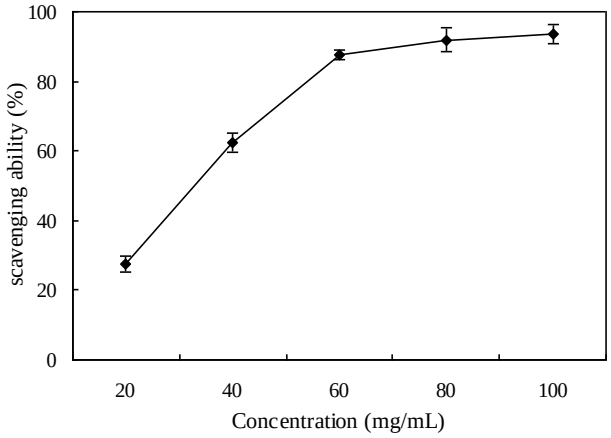

Figure 2. ABTS radicals scavenging activity of extracts

DIZ, MIC and MBC of the Extracts: The DIZ, MIC, and MBC values of the extracts from the radix of *Scutellaria baicalensis* Georgi are presented in Table 1. The results showed that the extracts had antibacterial activity against all of the tested foodborne pathogens, including

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both Gram-positive and Gram-negative bacteria. The DIZ values for all tested bacterial strains were in the range of 12.3-17.1 mm. The DIZ was the maximum value for *S. aureus*, followed by for *B. subtilis*. The MIC and MBC values for tested bacterial strains were in the range of 5-20 mg/mL and 10-40 mg/mL, respectively. Of Gram-positive bacteria, the extracts possessed the largest DIZ and lowest MIC and MBC values against *S. aureus*, which indicated that it was more effective bacterial inhibitor against *S. aureus*. From the result we can find that the Gram positive bacteria were more sensitive than the Gram-negative ones to the extracts, which was likely due to the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. The resistance of Gram-negative bacteria toward antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, almost impermeable to lipophilic compounds, presenting a barrier to the penetration of numerous antibiotic molecules (Nikaido 1994; Gao *et al*., 1999). While antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm, vital intracellular constituents or impairment of the bacterial enzyme systems because of the absence of this barrier in Gram-positive bacteria (Wendakoon & Sakaguchi 1995; Kalemba & Kunicka 2003).

Table 1. The DIZ, MIC, and MBC of the extracts from *Scutellaria baicalensis* Georgi radix

Bacteria	DIZ [a] (mm)	MIC (mg/mL)	MBC (mg/mL)
Gram-positive			
S. aureus	17.1±0.6	5	10
S. albus	14.8±0.4	10	20
B. subtilis	16.2±0.3	5	10
Gram-negative			
S. enterica	14.2±0.7	20	20
S. dysenteriae	12.3±0.6	20	40
E. coli	13.2±0.3	20	40

[a] Values represent means of three independent replicates ± SD. Different letters within a column indicate statistically significant differences between the means ($p < 0.05$) for DIZ.

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Kill-time analysis: Figure 3 showed that different concentrations extracts had significant effects on growth of *S. aureus.* The untreated *S. aureus* bacteria started to propagate rapidly and was entering the logarithmic phase after cultured for 2 h. Compared with this, S. *aureus* treated with extracts at the 0.5×MIC value showed a significant increase in the absorbance value of cultures at 600 nm (OD_{600}) until cultured for 6 h though its absorbance value was obviously much lower than that of control, which indicated that concentrations of the extracts lower than MIC did not completely inhibit growth of *S. aureus* but did increase the lag time in the growth curve (Figure 3). By contrast, the absorbance value of treatments at 1×MIC had no change during 9 h of incubation. These results confirmed the antibacterial activity of extracts and showed a severe effect on the growth rate of surviving *S. aureus*, supporting the results stated above, and showed that the treatment time and concentration of extracts had great influences on antibacterial effects.

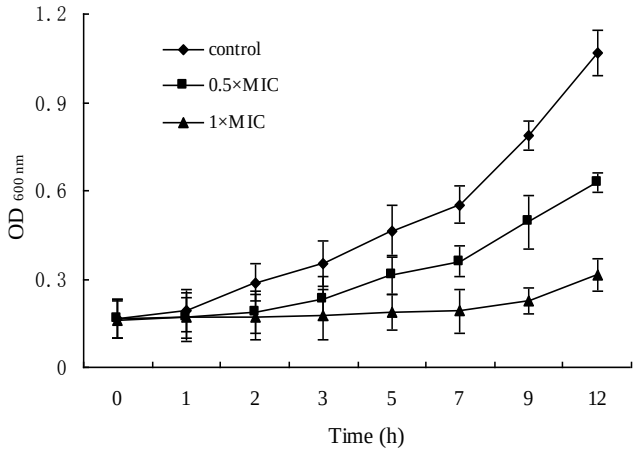

Figure 3. The effects of the extracts on the growth of *S. aureus*

CONCLUSIONS

This work showed that the extracts from the radix of *Scutellaria baicalensis* Georgi exhibited high effectiveness on scavenging free radicals, and the extracts showed varing degrees of antibacterial activity against tested Gram-positive bacteria and Gram-negative bacterial. However, further research on the chemical compositions, mechanisms of action, and the toxicological effect extracts from radix of *Scutellaria baicalensis* Georgi is still necessary to fully evaluate the potential of *Scutellaria baicalensis* Georgi in foods and medicines.

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